



INNOVATIONS FOR HEALTH

*The Use of Pharmacognosy and
Toxicology to Assess the Safety
of New Dietary Ingredients*

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- **What does reasonably expected to safe mean?**
- **When do we need toxicology studies?**
- **How do we decide which studies to conduct?**
- **How do we interpret and use these results?**
- **The role of pharmacognosy in safety assessment.**
- **How do we synthesize all the available information to determine safe use?**

- **Man in quest for food learned certain foods produced varying degrees of illness or death**
- **Soon recognized harmful & beneficial consequences associated with taking materials into his body**
- **Concept involving division of chemicals into two categories has persisted to the present day**
 - **Not possible, however, to describe a strict line of demarcation:**
 - **Beneficial chemicals**
 - **Harmful chemicals**
 - **Degrees of harmfulness & degrees of safeness for any chemical (the dose makes the poison)**
 - **All chemicals can cause toxic effects in large enough amounts**

- **Dose**
 - The amount of a substance that enters the body
- **Toxic**
 - Injurious to health or dangerous to life
- **Hazard**
 - Types of toxic effects caused by the chemical
 - Manifestation depends on route, amount, duration and frequency of exposure

- **Dose-response**
 - Quantitative relationship between dose and the magnitude of toxic response in the range of doses that might be or have been encountered
- **Risk**
 - Likelihood that the toxic properties of a chemical will be produced in populations of individuals under their actual conditions of exposure; exposure must precede adverse event
- **Safety**
 - Little or no harm will result from chemical under given set of exposure circumstances
 - It is not the absolute absence of risk; it is the inverse of risk

“The NDI safety standard is different than the standard for food additives, drugs, pesticides, and other FDA-regulated products. Recommendations in guidance documents that are tailored to the safety assessment needs of other FDA-regulated products may not always be appropriate for dietary ingredients and dietary supplements.”

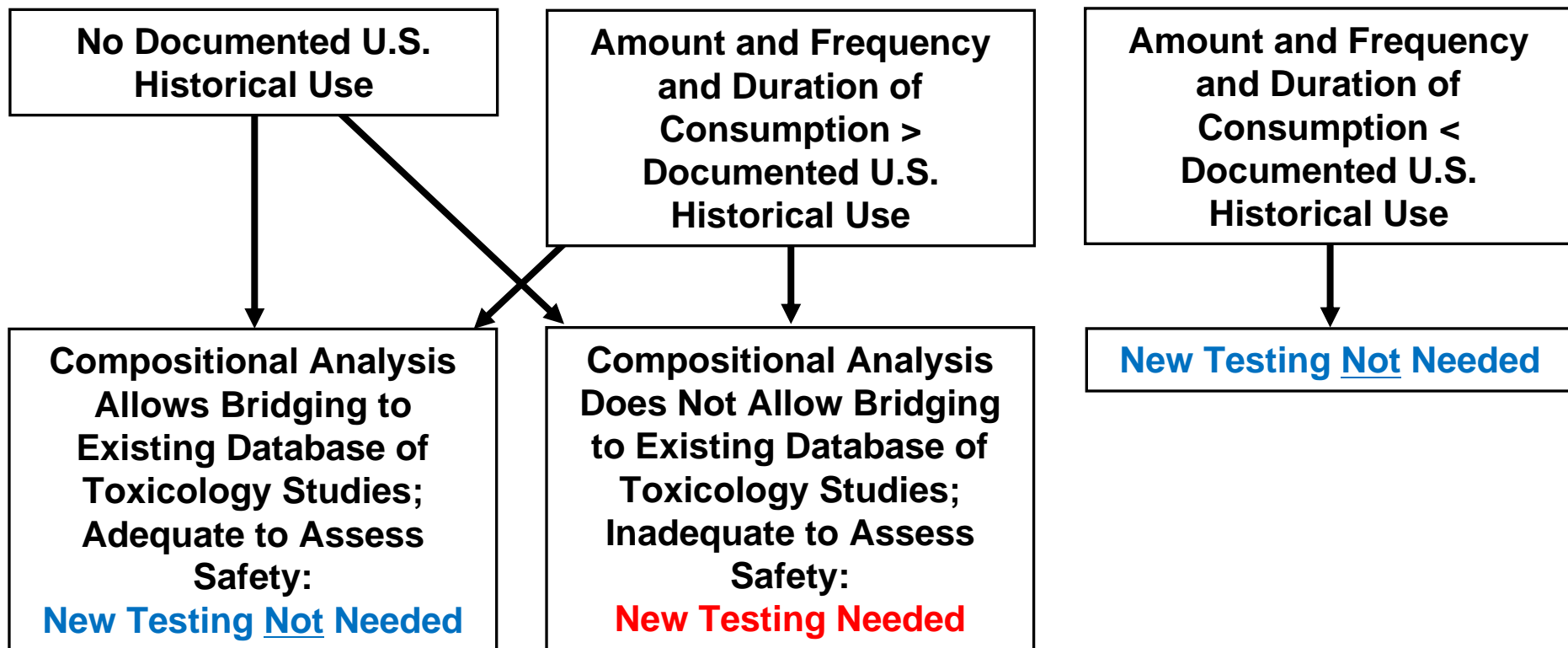
“You should use your own best judgment in compiling scientific evidence that provides a basis to conclude that the NDI that is the subject of your notification will reasonably be expected to be safe when used under the conditions recommended or suggested in the labeling of the dietary supplement described in the notification.”

- **To determine how an organism is affected by exposure to a substance**
 - **How the substance moves through the body**
 - **Metabolism of the substance**
 - **What organs or tissues are affected**
 - **The health outcomes of this exposure**
- **The more thorough this understanding, the more accurately we can predict what will happen when humans ingest the substance**

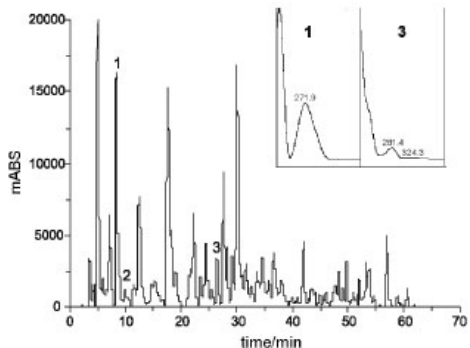
What Necessitates Toxicology Testing for an NDI?

- **Is there any historical use in the U.S.?**
- **Are current intake levels or recommended intake levels different from historical use?**
- **Is the current duration and frequency of use consistent with historical use?**
- **Is the current indication consistent with historical use?**
- **Has the target population changed?**
- **Has the traditional delivery matrix been altered or eliminated? (chemical or compositional change)**
- **If there are traditional cautions in the use of the NDI, are these cautions communicated to the consumer?**
- **Are there other reasons to expect a different toxicity profile for the proposed formulation versus the traditional preparations?**

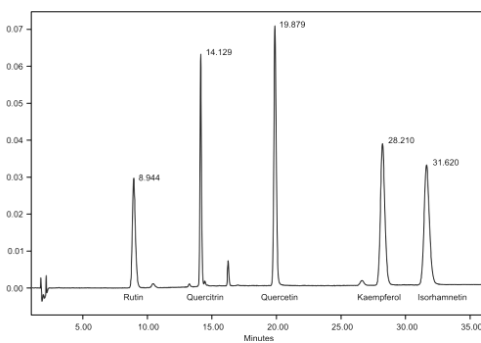
Decision Tree Approach for Toxicology Testing New Dietary Ingredient



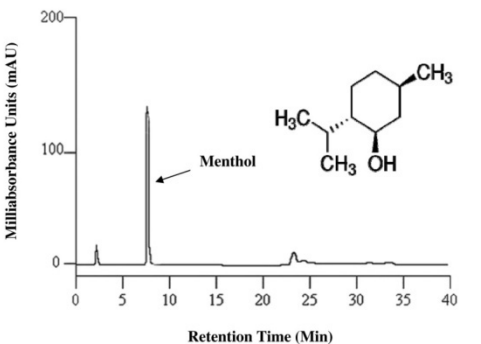
Pharmacognosy Establishes a “Chemical Bridge” to Information Used to Evaluate Safety of the NDI



◀ Extract



◀ Semi-purified fraction



◀ Purified compound



- **Chemically complex:** May be possible to bridge the NDI to historically consumed preparations, but only if both are well characterized. Preparations used in published toxicology studies may not be sufficiently well-characterized to enable their use for establishing safety by bridging to the NDI.
- **Chemically simple:** May not be possible to bridge the chemistry of the simpler NDI to “historical” preparations more inherently complex; however, may be possible to bridge the NDI to other substances that have safety data.

When we can't bridge the safety of the NDI for its intended use to documentation of historical use because of a change in:

- **Chemical composition**
- **Dose or amount ingested**
- **Duration of administration**
- **Frequency of administration**

Plant Source Material

➤ Species and Variety

- Any known adulterant or frequently substituted species?

➤ Plant part

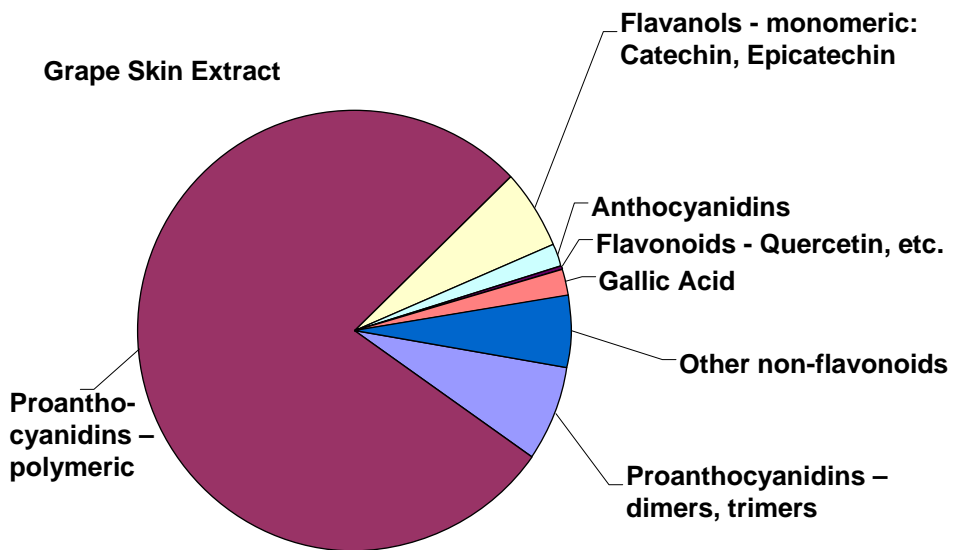
- Leaf, fruit, flowers, seed, stem, root, rhizome, total above ground parts

➤ Agricultural conditions, including country of origin

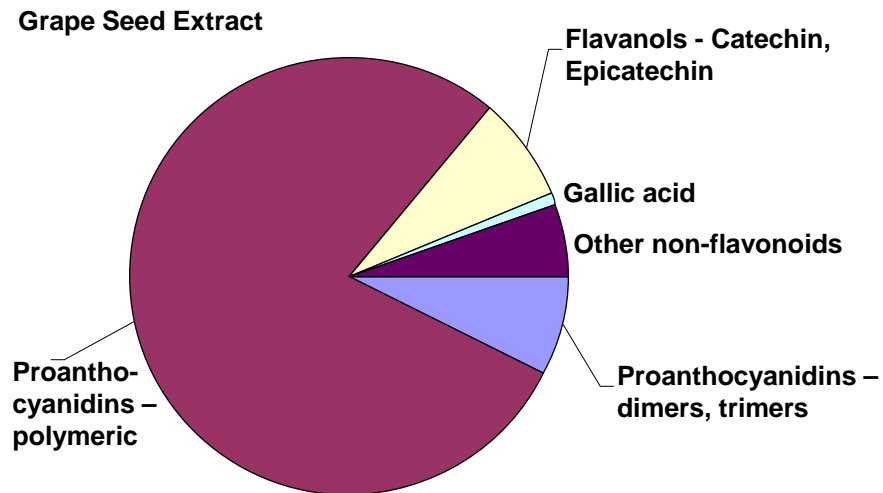
- Growing conditions: stressed plants produce more defense molecules
- Time of year to harvest: content of active(s), markers
- Pesticide/herbicide/insecticide application: chemical contamination
- Pollution: heavy metal content, etc.
- Harvesting and handling practices: mycotoxin content, mold, microbes, moisture

➤ Processing/Extraction Procedure

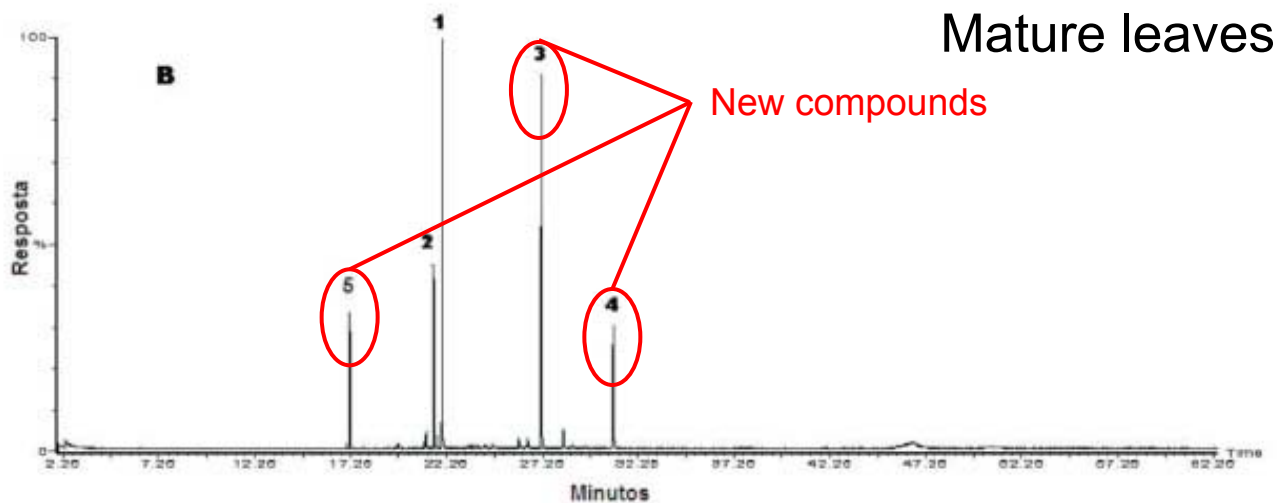
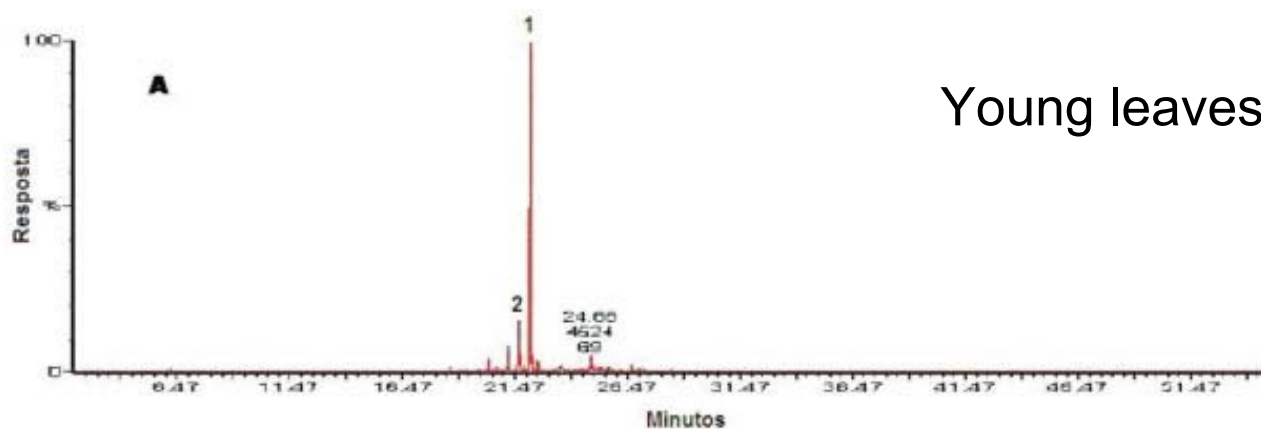
Polyphenols from Grape Skins



Polyphenols from Grape Seeds



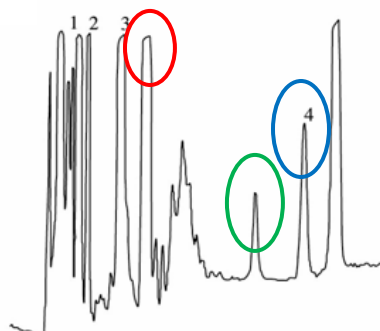
Change in Time to Harvest: Young vs. Old Leaves



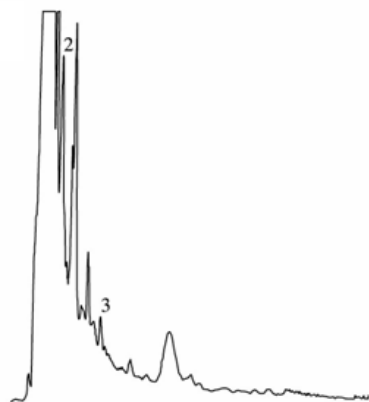
- **Extract vs. semi-purified fraction vs. pure compound?**
 - **Aqueous extract (tea or decoction)**
 - **Alcoholic extract (ethanol, isopropanol)**
 - **Oleoresin (hexanes, halogenated solvents, supercritical CO₂ extraction)**
 - **Essential oil (also present in oleoresins)**
 - **Semi-purified chromatographic fraction (“cleaner” than a crude extract but still contains multiple compounds)**
 - **Purified compound (single entity or racemic mixture of one molecule)**



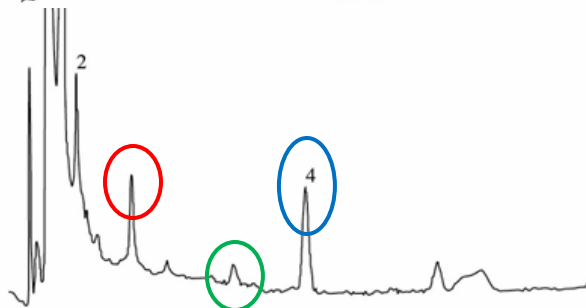
Extraction Method Produces Compositional Change



Fresh garlic



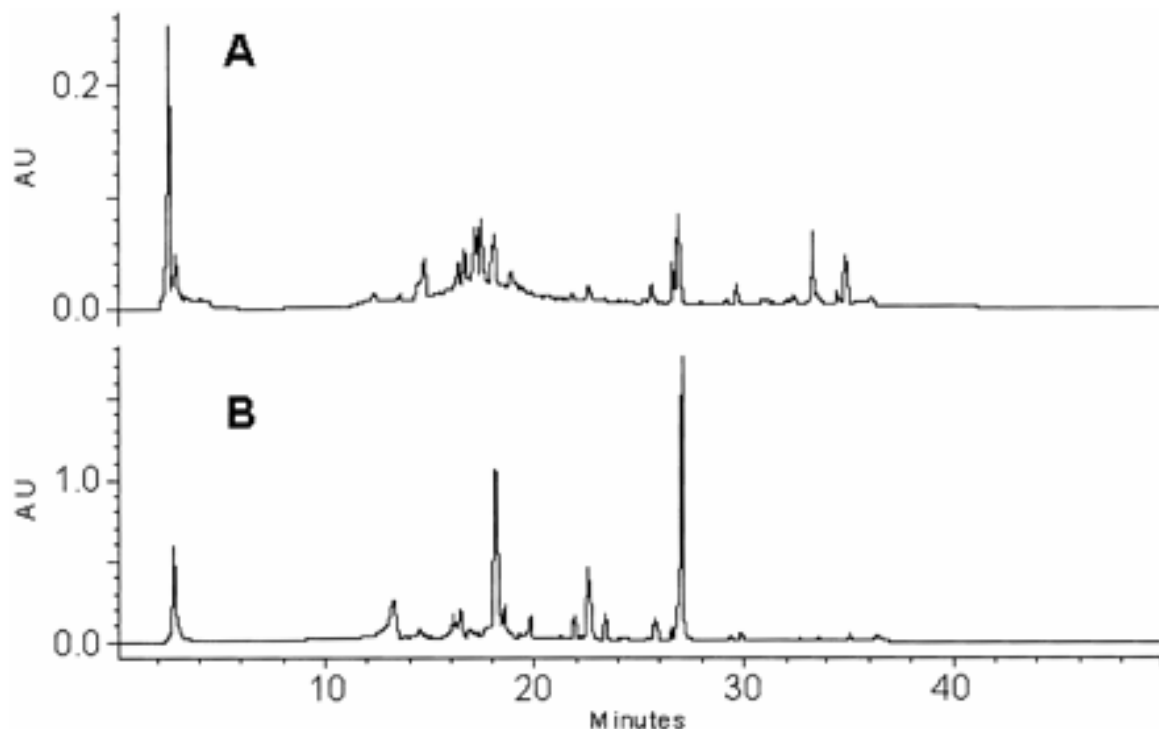
Ethanolic extract



Supercritical CO₂
(More like fresh garlic)

Questions:

- 1) Are extracts A & B *qualitatively* similar?
- 2) Are extracts A & B *quantitatively* similar?



Answers:

- 1) Yes.
- 2) No.

Is there a toxicological significance of the differences?

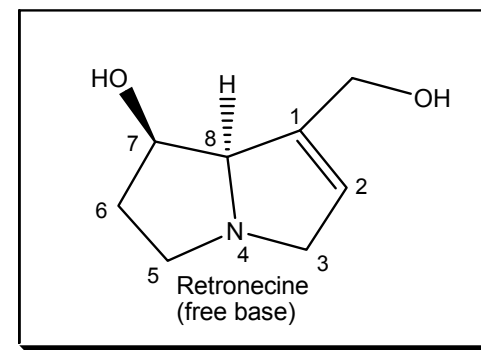
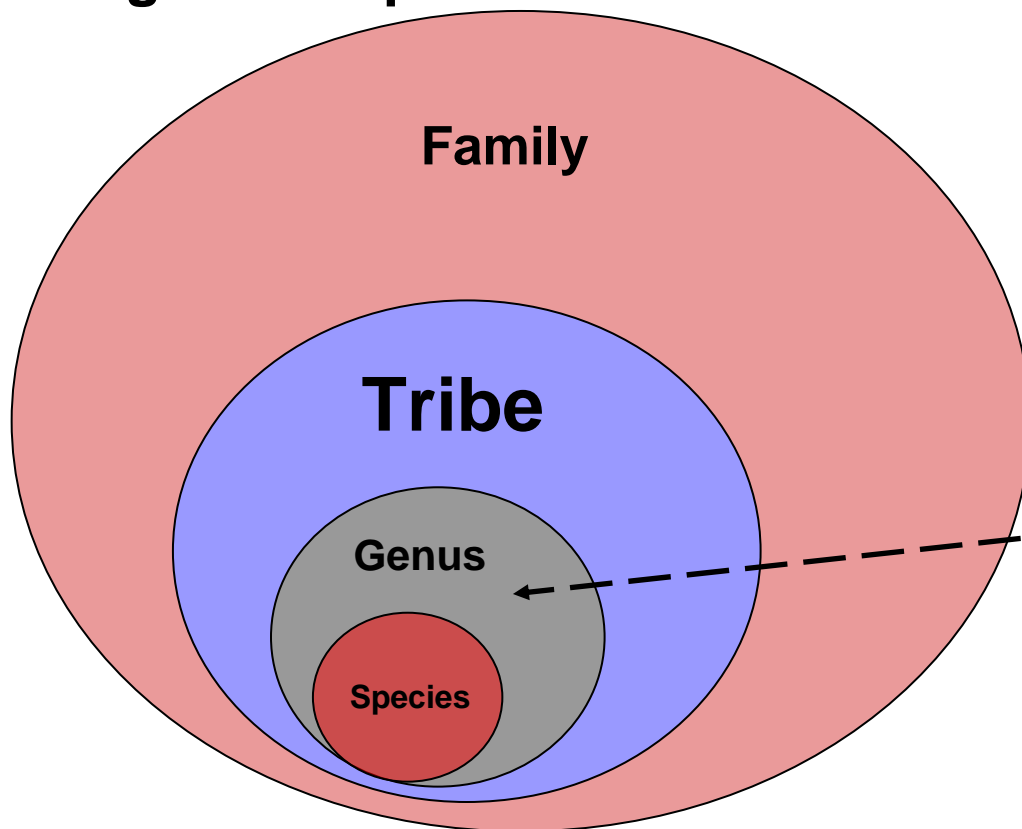
Predictive Role of Pharmacognosy

“What else could be in the extract?”

- **Are there (unreported) compounds or classes of compounds of concern present in the extract that we need to look for?**
- **Strategy:**
 - **Use plant taxonomy and correlative phytochemistry as predictive tools to determine possible occurrence**
 - **Perform chemical analyses for key compounds to fill data gaps**

Correlative Phytochemistry Can Help Predict Occurrence of Compounds of Concern

- Research compound occurrence reports for closely related taxa to assess the likelihood of compounds of concern occurring in the species of interest.



E.g., 1,2-unsaturated pyrrolizidine alkaloids (PAs) occurring in *Symphytum* and *Senecio* spp.

► **Avoid these genera of plants when formulating a botanical supplement, or analyze for PAs.**

We can't predict toxicity of the NDI for its intended use compared to historical use because of a change in:

- **Chemical composition**
- **Dose or amount ingested**
- **Duration of administration**
- **Frequency of administration**

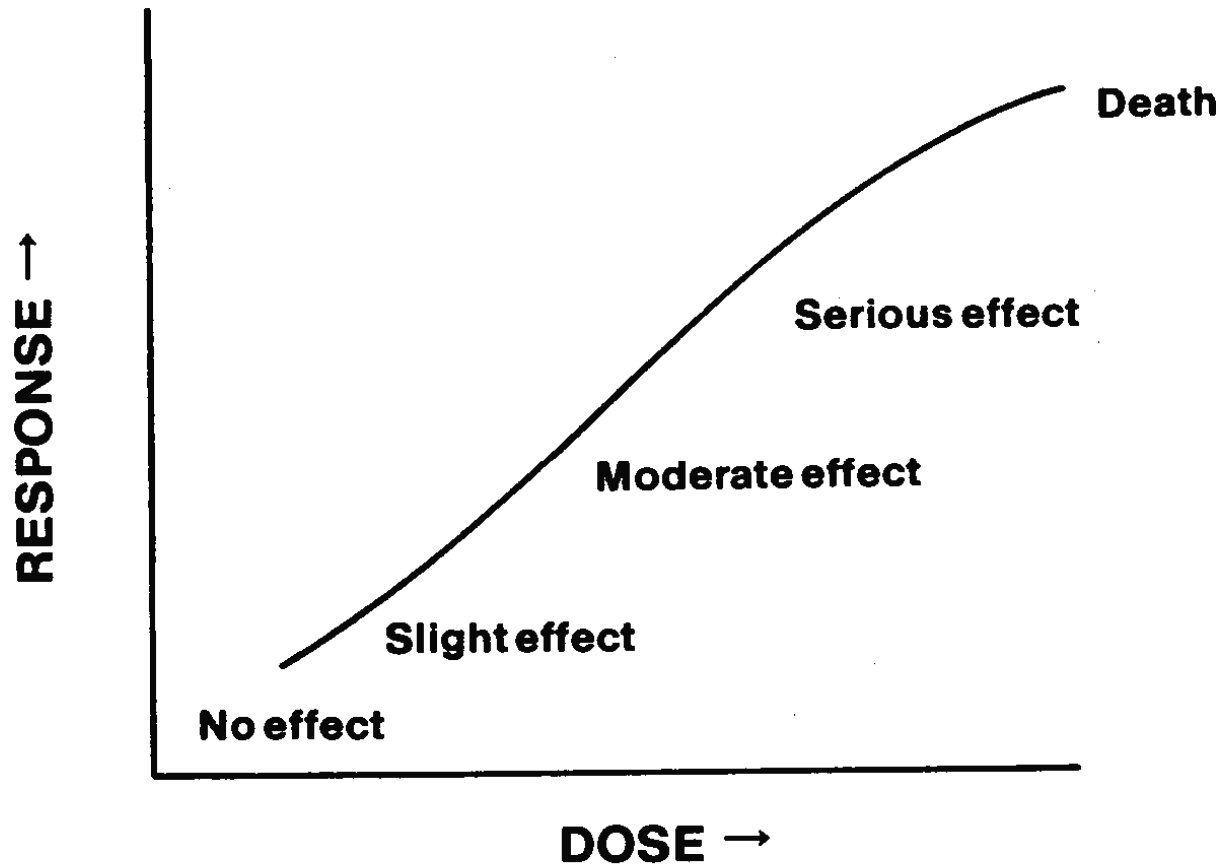
What Affects The Response to A Dose?

- **Dose Amount: How much?**
- **Dose Frequency: How often?**
- **Dose Duration: How long?**

“All things are poison and nothing is without poison, only the dose permits something not to be poisonous.”

The Central Tenet of Toxicology:

It Is the Dose That Makes the Poison



Dose-response relationship for a typical chemical.

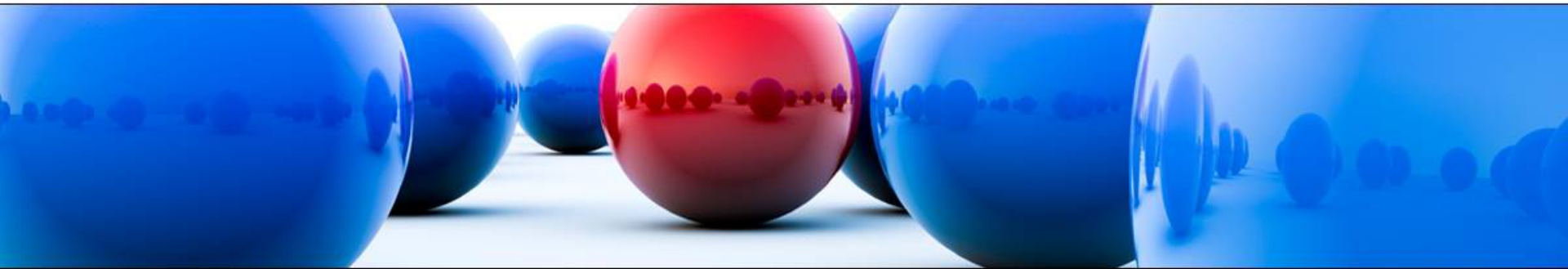
We can't predict toxicity of the NDI for its intended use compared to historical use because of a change in:

- **Chemical composition**
- **Dose or amount ingested**
- **Duration of administration**
- **Frequency of administration**

- **Efficiency of repair is an important determinant of the dose-response relationship**
 - **Amount, frequency and duration of exposure are involved**
 - **For example, repair processes may not be overwhelmed at a dose given over a short period of time but the same dose given over a longer period of time may overwhelm these repair processes, resulting in toxicity**
 - **Similarly, frequency of exposure of the same dose may affect the efficiency of the repair processes, producing more toxicity at greater frequencies of administration**

Animal Toxicology

How do we decide which studies to carry out?



- **Animal toxicology is a tool: classic rodent studies evaluate toxicity**
- **Animal models must be chosen appropriately to extrapolate to the human, including consideration of:**
 - **Bioavailability**
 - **Nutritional requirements/limitations**
 - **Metabolic parameters**
 - **Developmental stage**
- **Study must be designed to prevent differences in pharmacokinetic handling or dietary imbalance from confounding toxicology results**
- **Strengths**
 - **Well controlled experiments, controlled doses, no confounding exposures issues**

Toxicology Testing Can Inform Us About:

- **Hepatotoxicity**
- **Nephrotoxicity**
- **Cardiovascular toxicity**
- **Pulmonary toxicity**
- **Dermal toxicity**
- **Ocular toxicity**
- **Developmental toxicity**
- **Neurotoxicity**
- **Behavioral toxicity**
- **Immunotoxicity**
- **Hematopoietic toxicity**
- **Reproductive toxicity**
- **Endocrine organ toxicity**
- **Gastrointestinal toxicity**

- **Local and systemic effects**
 - At site of first contact (gastrointestinal)
 - At site(s) distal to point absorbed (internal organ damage)
- **Reversible and irreversible effects**
 - Disappear following cessation of exposure (enzyme changes, respiratory depression)
 - Persist or even progress after exposure is discontinued (cancer, genetic alterations, birth defects, death)
- **Immediate and delayed effects**
 - Develop shortly after single exposure (cyanide poisoning)
 - Occur after a lapse of time (10-20 years for cancer)

Frequency and Duration of Exposure to the NDI Determines the Study Needed

- **Chronic Use:** long-term use, assumed to be consumption every day throughout life
 - **Daily Use:** ingestion at least once a day, every day, for at least three months in a row or for more than 90 days in a year
- **Intermittent Use:** any use that is less frequent than daily use
 - **Subchronic Use**
 - Daily and finite
 - Non-daily and lifetime

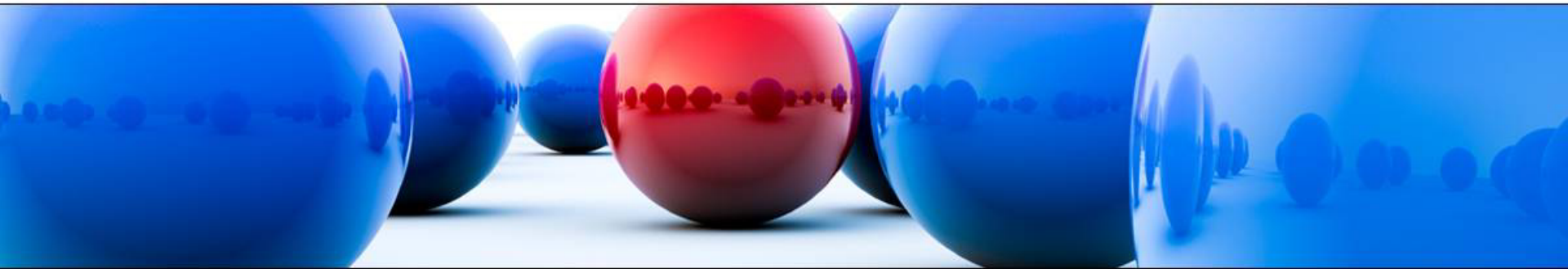
“USE DAILY MEANS LIFETIME”

Safety Testing Recommendations

Draft Guidance

- **Genotoxicity Battery**
 - **Bacterial mutagenesis, *in vitro* cytogenetics, *in vivo* mammalian test**
- **Repeat dose toxicity**
 - **14-day Range-Finding**
 - **90-Day Subchronic**
- **Chronic/Carcinogenicity**
- **Reproductive**
 - **One generation; Multi-generation**
- **Developmental/Teratology**
- **ADME**

How Do We Interpret and Use These Results To Assess Safety



Animal Toxicology is Used to Derive an ADI

- **Acceptable Daily Intake (ADI) is defined as the daily intake of the NDI that during the human lifetime appears to be without appreciable risk.**
- **Risk is the likelihood that toxicity will be produced under the conditions of exposure.**
- **Safety is the inverse of risk.**
- **Safety for an NDI is defined as the reasonable expectation of safety under the conditions of use.**

Derivation of the ADI to Support Safe Intake of the NDI

$$\text{ADI} = \frac{\text{NOAEL (mg/kg/day)}}{\text{Safety Factors}}$$

ADI = Acceptable Daily Intake

- **NOAEL**: the No-Observable-Adverse-Effect Level which is the highest dose that did not elicit an adverse effect in a properly designed and executed toxicology study
- **NOEL**: the No-Observable-Effect Level which is the highest dose at which no effects (beneficial, neutral or adverse) are observed in a properly designed and executed toxicology study

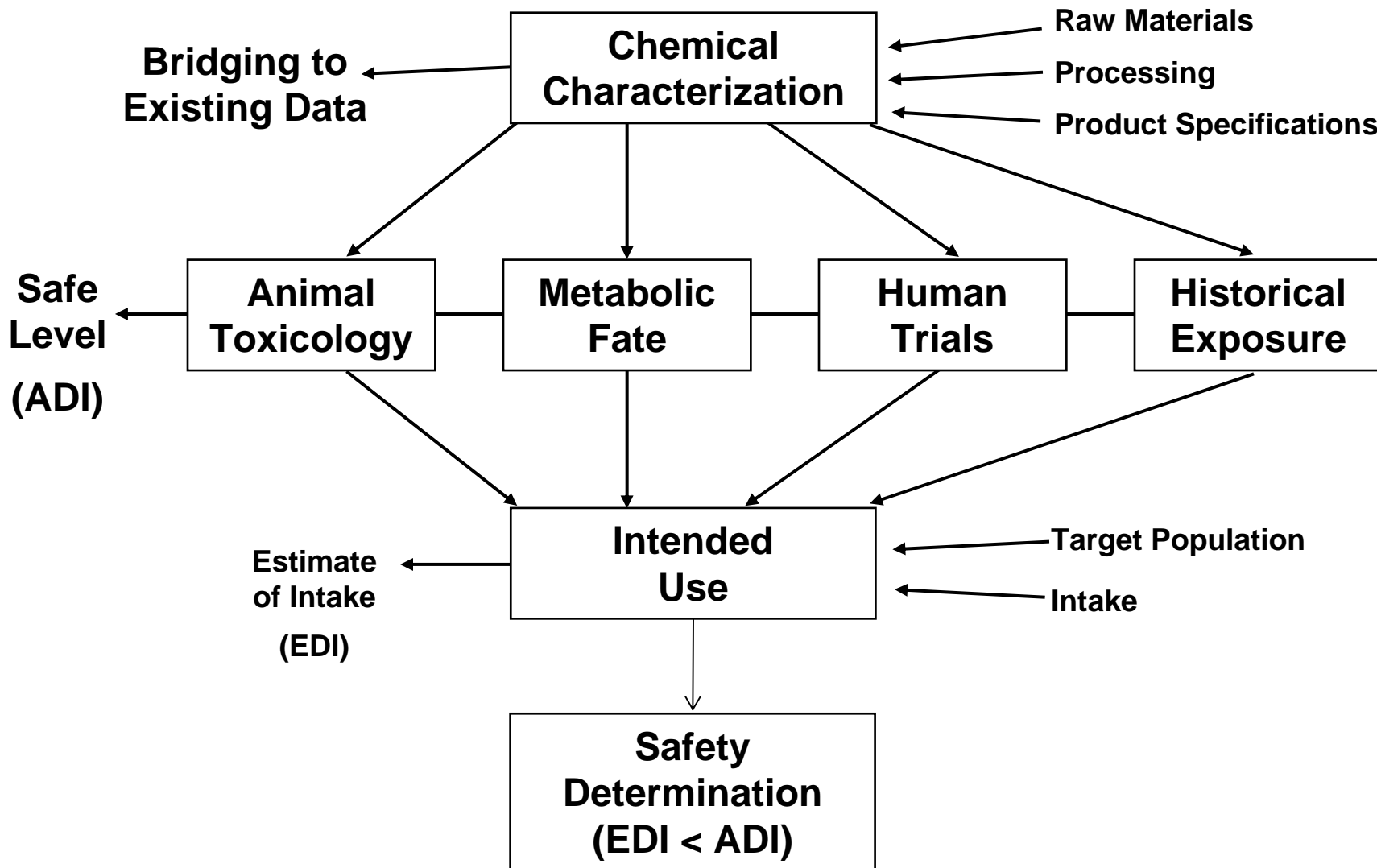
- **Safety Factor or Uncertainty Factor:**
 - **a multiplier used to account for differences between animals and humans, between differences in humans, and limitations in animal studies that allows us to deal with the uncertainty about the predictive value of the animal data to extrapolate to humans in the context of safety**

What Changes the Sensitivity of a Response to a Dose

- **Interspecies Variation**
 - **Animals → Humans**
- **Intraspecies Variation: Human variability**
 - **Health status**
 - **Body weight**
 - **Age**
 - **Sensitivity**

- **Intraspecies (10 X):**
 - **May be smaller when there is a long history of food use by a large, diverse population. Factor should be large when toxicity is severe or studies have limited duration or small populations**
- **Interspecies (10 X)**
- **Subchronic to chronic (10 X):**
 - **If only one subchronic study is available an additional factor of 2 may be used**

Safety Analysis



Thank You From the Spherix Team

- US Based:

- Claire Kruger, PhD, DABT
- A. Wallace Hayes, PhD, DABT
- Nancy Booth, PhD
- Ronald Slesinski, PhD, DABT
- Susan Phillips, MS
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- Roger Clemens, PhD, CNS
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- Robert Lodder, PhD

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- Nino Binns, PhD – EU
- Silvia Berlanga de Moraes Barros, PhD – Latin America
- Tetsuo Satoh, PhD – Japan
- S.K. Gupta, PhD, DSc – Asia
- Govinder Flora, PhD – Asia

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